

Amendments to the Claims

The listing of claims will replace all prior versions, and listings of claims in the application.

1. (currently amended) A method for obtaining human erythropoietin comprising

(a) culturing mammalian cells which express recombinant human erythropoietin in culture medium consisting of :

(i) DMEM (Dulbecco's modified Eagle's medium) [5] ;

(ii) F12 medium [5] ;

(iii) insulin₁ and ~~an additive, wherein the additive is selected from the group consisting of~~

(iv) NaHCO₃, sugars, ethanolamine, pyruvate, and amino acids as additives and mixtures thereof ; and

(b) obtaining human erythropoietin from said culture medium.

2. (previously presented) The method of claim 1, wherein said cells are selected from the group consisting of CHO, COS, BHK, Namalwa, and HeLa.

3. (original) The method of claim 2, wherein said cells comprise CHO cells.

4. (previously presented) The method of claim 1, wherein said culture medium comprises greater than about 1 mg insulin per liter of culture medium.

5. (previously presented) The method of claim 1, wherein said culture medium comprises less than about 20 mg insulin per liter of culture medium.

6. (canceled)

7. (previously presented) The method of claim 1, wherein said obtaining comprises:

- (i) separating supernatant comprising EPO and insulin from said cells;
- (ii) concentrating supernatant of step (i); and
- (iii) freezing concentrated product of step (ii).

8. (previously presented) The method of claim 7, wherein media is added to separated cells of step (i) and said cells are cultured.

9. (previously presented) The method of claim 7, wherein supernatant of said step (i) is concentrated from about 50 to 150 fold.

10. (previously presented) The method of claim 7, wherein supernatant of said step (i) is concentrated about 100 fold.

11. (previously presented) The method of claim 7, wherein said step (ii) comprises using a tangential filtration system through membranes with a molecular weight cut-off of about 3,000 Daltons.

12. (previously presented) The method of claim 7, further comprising (iv) sterile filtering the concentrated product of step (iii) through membranes with pores of diameters of about 0.2 μm .

13. (previously presented) The method of claim 1, wherein said culture medium comprises about 10 mg insulin per liter of culture medium.

14. (canceled)

15. (previously presented) The method of claim 1, wherein said sugars are selected from the group consisting of glucose, lactose, galactose and mixtures thereof.

16. (previously presented) The method of claim 1, wherein said pyruvate is sodium pyruvate.

17. (previously presented) The method of claim 1, wherein said amino acids are selected from the group consisting of glutamine, tryptophan, asparagine, serine and mixtures thereof.

18. (canceled)

19. (currently amended) The method of claim 1 18, wherein said culture medium contains ~~Iscove's DMEM, HAM's F12 medium, insulin and~~ NaHCO₃, glucose,

lactose, galactose, ethanolamine, sodium pyruvate, glutamine, tryptophan, asparagine and serine as additives.

20. (previously presented) The method of claim 1, wherein said DMEM is Iscove's DMEM and wherein said F12 medium is HAM's F12 medium.

21. (New) A method for obtaining human erythropoietin comprising
(a) culturing mammalian cells which express recombinant human erythropoietin in culture medium consisting of:

- (i) DMEM (Dulbecco's modified Eagle's medium);
- (ii) F12 medium;
- (iii) insulin; and
- (iv) NaHCO_3 , glucose, lactose, galactose, ethanolamine, sodium pyruvate, glutamine, tryptophan, asparagine and serine as additives; and

(b) obtaining human erythropoietin from said culture medium.

22. (New) The method of claim 21, wherein said cells are CHO cells.